American Journal of
EPIDEMIOLOGY

Volume 156

Number 2

July 15, 2002

Sponsored by the Society for Epidemiologic Research
Published by Oxford University Press

HUMAN GENOME EPIDEMIOLOGY (HuGE) REVIEW

Pooled Analysis and Meta-analysis of Glutathione *S*-Transferase M1 and Bladder Cancer: A HuGE Review

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Received for publication July 25, 2001; accepted for publication March 20, 2002.

Smoking is a known risk factor for bladder cancer. The product of the *GSTM1* gene, glutathione *S*-transferase M1 (GSTM1), is involved in the detoxification of polycyclic aromatic hydrocarbons found in tobacco smoke; a homozygous deletion of this gene in approximately 50% of Caucasians and Asians results in a lack of GSTM1 enzyme activity. Most studies examining the relation between bladder cancer and GSTM1 have reported an increased risk associated with a lack of GSTM1 activity. The authors performed meta- and pooled analyses of published and unpublished, case-control, genotype-based studies that examined this association (17 studies, 2,149 cases, 3,646 controls) and excluded studies conducted in populations with a high prevalence of exposure to known bladder cancer risk factors other than tobacco smoke. Using random effects models in the meta-analysis, the authors obtained a summary odds ratio of 1.44 (95% confidence interval (CI): 1.23, 1.68) for *GSTM1* null status with all studies included. Results from studies with at least 100 cases and 100 controls produced a summary odds ratio of 1.42 (95% CI: 1.26, 1.60). Pooled analyses using original data sets from 10 studies (1,496 cases and 1,444 controls) and adjusting for age, sex, and race produced similar results. There was no evidence of multiplicative interaction between the *GSTM1* null genotype and ever smoking in relation to bladder cancer, although there was a suggestion of additive interaction (additive interaction = 0.45, 95% CI: –0.03, 0.93). These results indicate that, among populations studied to date, *GSTM1* null status is associated with a modest increase in the risk of bladder cancer. *Am J Epidemiol* 2002;156:95–109.

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bladder neoplasms; epidemiology; genetics; glutathione transferase

Abbreviations: CI, confidence interval; CYP, cytochrome P450; GST, glutathione *S*-transferase; GSTM1, glutathione *S*-transferase M1; OR, odds ratio.

GENE

The glutathione *S*-transferases (GSTs) are a family of enzymes that are important in the metabolism of a wide variety of xenobiotics, including environmental carcinogens, reactive oxygen species, and chemotherapeutic agents (1). They act as phase II metabolizing enzymes, catalyzing reactions between glutathione and various electrophilic compounds. Five GST enzyme classes (α, γ, θ, μ, and π) have been identified in humans. Because of their detoxification role, these enzymes and the genes encoding them may play an important role in cancer susceptibility. Although the vast majority of reactions catalyzed by the GSTs result in detoxification products, there are a few cases in which the reaction is reversible or in which the product, or a metabolite of the product, is more reactive than the parent compound (1).

The *GSTM1* gene codes for the cytosolic enzyme GST-μ. This enzyme has received considerable attention in relation to bladder cancer and other smoking-related cancers because of its role in the detoxification of benzo[*a*]pyrene and other polycyclic aromatic hydrocarbons found in tobacco smoke. Three polymorphisms of the *GSTM1* gene, which is located on chromosome 1p13.3, have been identified. One polymorphism is a deletion that results in a lack of functional gene product (*GSTM1-0*). The other two (*GSTM1a* and *GSTM1b*) differ by a C→G substitution at base position 534, resulting in a Lys→Asn substitution at amino acid 172 (2). Because there is no evidence of functional difference between *GSTM1a* and *GSTM1b*, the two are typically categorized together as a single functional phenotype.

Persons with homozygous deletion of *GSTM1* (*GSTM1* null) show no GST-μ activity. Several studies have shown high agreement (i.e., >94 percent) between the *GSTM1* genotype and the GST-μ phenotype (3–7). Most studies of *GSTM1* and cancer have compared the homozygous deletion genotype with the genotypes containing at least one functional allele.

GENE VARIANTS

The distribution of the *GSTM1* null genotype among ethnic groups has been reviewed extensively by Cotton et al. (8) and Geisler and Olshan (9) and will be described only briefly here. In the United States, the frequency of the *GSTM1* null genotype varies across ethnic groups (8). The mean reported frequency is 29 percent (95 percent confidence interval (CI): 26, 32) among persons of African descent, 50 percent (95 percent CI: 45, 55) among persons of Asian descent, 46 percent (95 percent CI: 41, 52) among persons of Hispanic descent, and 51 percent (95 percent CI: 49, 52) among persons of European descent.

The mean frequencies of the *GSTM1* null genotype among the ethnic groups listed above are very similar to the frequencies observed in those groups' places of origin (2, 8, 9). The highest frequencies of 64–100 percent have been observed among small samples of Pacific Islanders; these estimates were obtained using Southern blot while most others were obtained via polymerase chain reaction. The estimates presented here are very similar to estimates based on control distributions in a database of results from studies of metabolic gene polymorphisms maintained by the International Collaborative Study on Genetic Susceptibility to Environmental Carcinogens, which also includes unpublished data (10).

DISEASE

Incidence and mortality rates of bladder cancer vary about 10-fold worldwide (11–13). The highest rates are found in North America and Europe, although the rates in eastern Europe tend to be lower than elsewhere in Europe. Rates are low in many parts of Asia. Differences in pathologic classification of bladder tumors, especially the registration of "benign" tumors or "papillomas" as cancer in some countries, may explain some of the geographic variation in rates; however, evidence suggests that this plays a relatively small role in the observed patterns (14–17).

Internationally, transitional cell carcinomas account for about 95 percent of bladder neoplasms (14, 15). The remaining 5 percent consist of squamous cell carcinomas and adenocarcinomas, with squamous cell carcinomas more common. Most registries and national statistics do not distinguish among different bladder tumor types, making comparisons of rates difficult. However, limited evidence suggests that the proportion of bladder cancers that are squamous cell is higher in countries with endemic schistosomiasis (i.e., infection by *Schistosoma hematobium*) (18–20). Bladder cancer also tends to occur at a younger age in these countries, with many cases being diagnosed when they are between 40 and less than 60 years of age (14). Information is lacking on the incidence and mortality of bladder adenocarcinoma.

In the United States, bladder cancer incidence varies markedly by race (21). African Americans and Hispanic Whites have incidence rates approximately half those of non-Hispanic Whites. Rates are even lower among Asian Americans; among persons of Filipino descent, they are about one-quarter that of non-Hispanic Whites.

Males consistently show a higher incidence than do females throughout the world, with male/female ratios varying from approximately 2.5 to 5 (12, 15, 17). In the United States, the ratio of males to females is about 2.6

among African Americans, 3.3–3.7 among Hispanic Whites and most Asian groups, and 4.0 among non-Hispanic Whites and Filipinos (22). This gender disparity may be partially due to historical differences between males and females in occupational exposures and cigarette smoking behaviors.

The incidence and mortality of bladder cancer rise exponentially with age, with very few cases less than the age of 30 years and about two thirds of cases occurring over the age of 65 years (12). Both gender differences and histologically high-grade tumors are strongly associated with increasing age (15).

Age-adjusted incidence rates have been rising in many parts of Europe and North America over the last few decades (12, 14). In contrast, rates have remained relatively constant in Asia and Australia. Increases have generally been greater among males than among females. These trends may reflect changing geographic and gender patterns of smoking.

Cigarette smoking is a well-documented risk factor for bladder cancer (12, 14, 23). In fact, although certain occupational exposures such as aromatic amines present greater relative risks of bladder cancer, cigarette smoking has a greater population attributable risk because of its higher prevalence (23). An estimated 66 percent of bladder cancers in Western countries is attributable to tobacco smoking (23). This increased risk is probably due to the presence in cigarette smoke of polycyclic aromatic hydrocarbons, nitrosamines, and aromatic amines. Smokers have 2.5–3.5 times the risk of bladder cancer compared with nonsmokers. Dose-response relations have been observed for both the intensity (i.e., number of cigarettes smoked per day) and the duration of smoking, although the relation for intensity appears to level off at higher exposures (24). Quitting smoking reduces bladder cancer risk, although former smokers continue to be at increased risk relative to never smokers (25, 26). Smokers of black tobacco have 2–3 times the risk of bladder cancer than do smokers of blond tobacco (27–33). This is probably due to the higher levels of aromatic amines found in black tobacco. The evidence is inconsistent for increased risk of bladder cancer related to other forms of tobacco use, although there is the suggestion of an association with pipe smoking (12, 14, 26).

Bladder cancer is associated with a number of occupations or occupational exposures. The first such association was observed in 1895 by Rehn (34), who reported high rates of bladder cancer among men employed in the aniline dye industry. Subsequent research among dyestuffs workers identified the aromatic amines benzidine and 2-naphthylamine, and possibly 1-naphthylamine, as bladder carcinogens (35). Since then, these and several other aromatic amines and related compounds have been identified as either likely or suspected human bladder carcinogens (36–46). These include 4-aminobiphenyl, 4-chloro-*o*-toluidine, *o*-toluidine, 4,4′-methylenedianiline, and 4,4′-methylene-bis(2-chloroaniline). An excess risk of bladder cancer has also been observed among rubber workers (with possible exposure to 2-naphthylamine or a precursor) (47–49); painters (potentially exposed to dyes, polychlorinated biphenyls, formaldehyde, asbestos, and solvents) (50–59); truck, bus, and taxi drivers (probably exposed to polycyclic aromatic hydrocarbons and nitro-polycyclic aromatic hydrocarbons in automobile exhaust) (30, 55, 60–70); aluminum workers (possibly from exposure to coal-tar pitch volatiles) (71–76); and leather workers (with possible exposure to benzidine-based azo dyes, aromatic solvents, formaldehyde, chromium/chromate, and leather dust) (53, 61, 77–81).

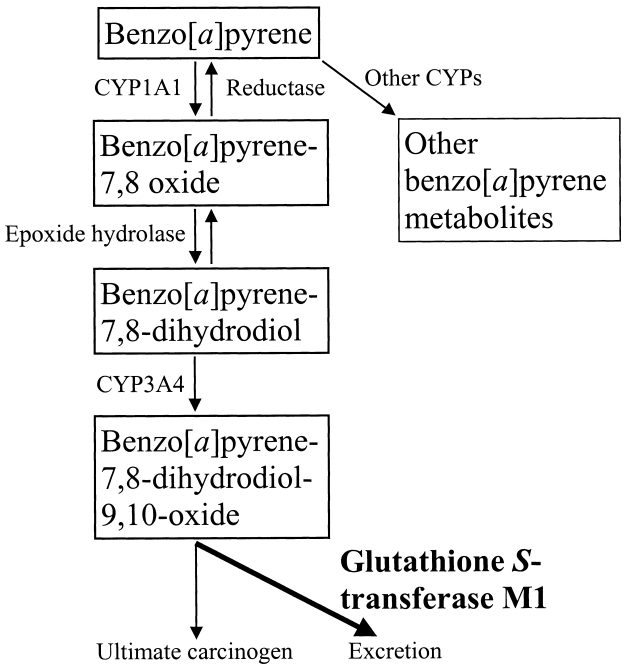

FIGURE 1. Role of glutathione *S*-transferase M1 (GSTM1) in the detoxification of benzo[*a*]pyrene. CYP, cytochrome P450.

Other known or suspected risk factors for bladder cancer include chlorination by-products in drinking water (82–84) and arsenic (often through drinking water) (85, 86), ionizing radiation (87, 88), bladder infection (89–91), high consumption of phenacetin-containing analgesics (92, 93), and hair dyes (12). Most studies suggest a causal relation between *S. hematobium* and bladder cancer (94–96).

Several genetic susceptibility factors have been examined in relation to bladder cancer. Polymorphisms in the *N*-acetyltransferase 2 gene (*NAT2*), which is involved in the phase II detoxification of aromatic amines, appear to affect bladder cancer risk, with slow acetylators having modestly increased risk (97–100). There is also evidence for a multiplicative interaction between smoking and *NAT2* (98). Glutathione *S*-transferase M1 (GSTM1), involved in the phase II detoxification of polycyclic aromatic hydrocarbons (figure 1), has been studied in relation to bladder cancer in two previous meta-analyses (100, 101) and is examined in greater detail below. There is a suggestion of increased risk of bladder cancer among subjects with high activity of a cytochrome P450 (CYP) enzyme involved in the metabolic activation of aromatic amines (CYP1A2) (102). Inconsistent results have

TABLE 1. Source and description of data used in the pooled and meta-analyses of glutathione *S*-transferase M1 (*GSTM1*) null status and bladder cancer risk

Author(s) (reference), year	No. of cases*	No. of controls*	Country	Control source	OR†	95% CI†
Brockmöller et al. (118), 1996‡	374	373	Germany	Hospital	1.29	0.97, 1.72
Okkels et al. (123), 1996‡	234	202	Denmark	Hospital	1.34	0.92, 1.96
Bell et al. (116), 1993‡	229	211	United States	Hospital	1.68	1.15, 2.46
Kang et al. (128), 1999‡	174	147	Korea	Hospital	1.64	1.05, 2.57
Schnakenberg et al. (126), 2000	157	223	Germany	Population	1.06	0.70, 1.60
Peluso et al. (124), 2000‡	130	54	Italy	Hospital	0.76	0.40, 1.44
Lin et al. (117), 1994‡	114	1,104	United States	Hospital, population	1.29	0.88, 1.91
Kim et al. (130), 2000	112	220	Korea	Hospital	1.81	1.12, 2.93
Katoh et al. (129), 1998‡	112	112	Japan	Hospital	1.78	1.05, 3.02
Zhong et al. (127), 1993	97	225	United Kingdom	Hospital, population	0.94	0.58, 1.52
Chern et al. (119), 1994‡	95	74	United Kingdom	Population	1.61	0.87, 2.99
Georgiou et al. (121), 2000	89	147	Greece	Hospital	2.76	1.60, 4.75
Šalagovic et al. (125), 1999‡	76	248	Slovakia	Hospital	1.13	0.68, 1.89
Mungan et al. (122), 2000	61	69	Netherlands	Hospital	2.15	1.06, 4.34
Daly et al. (120), 1993‡	53	52	United Kingdom	Hospital	3.81	1.50, 9.70
Heckbert et al. (115), 1992‡,§	29	114	United States	Population	1.07	0.47, 2.44
Romkes, unpublished data‡,¶	13	71	United States	Population	1.47	0.44, 4.93
Daly, unpublished data‡,¶,#	241	0	United Kingdom			
Total in meta-analyses	2,149	3,646				
Total in pooled analyses	1,935	1,444				

* The numbers in the table are from the manuscripts or abstracts. For data sets with a different number of subjects, the numbers are as follows: Okkels et al., 254 cases and 179 controls; Kang et al., 232 cases and 165 controls; Peluso et al., 148 cases and 104 controls; Lin et al., 114 cases and 0 controls; Katoh et al., 65 cases and 101 controls; and Šalagovic et al., 88 cases and 0 controls.

† OR, odds ratio; CI, confidence interval. These 95% CIs are based on the variance estimate method of Woolf (150) and may differ slightly from the 95% CIs presented in the original manuscripts.

‡ Included in pooled analyses.

§ Included only genotyped subjects from the original study.

¶ Unpublished data of M. Romkes (University of Pittsburgh, Pittsburgh, PA) and A. K. Daly (University of Newcastle upon Tyne, Newcastle upon Tyne, England).

Included only in pooled case-only analyses, as described in the text.

been observed for CYP2D6 (16), while no association has been observed for CYP2E1 (12, 103, 104).

ASSOCIATIONS AND INTERACTIONS

To examine the association between *GSTM1* and bladder cancer, we undertook meta- and pooled analyses of all identified studies. To identify articles or abstracts containing information on *GSTM1* status and bladder cancer, we used review papers (2, 100, 105, 106), searched MEDLINE (using the keywords "glutathione *S*-transferase," "gstm1," and "bladder cancer") without restriction on language, and searched abstracts in the *Proceedings of the American Association for Cancer Research*. Eligible studies were those that used a case-control design, were population or hospital based, and assessed *GSTM1* status via genotype (table 1). We excluded studies conducted in populations with a high prevalence of exposure to known bladder cancer risk factors other than tobacco smoke, including schistosomiasis infection (107–109) and occupational exposure to aromatic amines and polycyclic aromatic hydrocarbons (110, 111). We also excluded phenotype-only studies (112) to reduce possible misclassification of *GSTM1* status (table 2). Although cigarette smoking probably contributed to the elevated risks of bladder cancer reported in the excluded studies, our goal was to focus on tobacco smoke as the primary known risk factor and, more specifically, on a particular class of carcinogen found in tobacco smoke, polycyclic aromatic hydrocarbons, which are detoxified by GSTM1. We then attempted to obtain the original data from all eligible studies published in manuscript or abstract form through May 2000 in one of three ways. First, we included all relevant data that had been previously contributed to a database maintained for such purposes by the International Collaborative Study on Genetic Susceptibility to Environmental Carcinogens (113). These data included, for each

TABLE 2. Source and description of data excluded from this study

Author(s) (reference), year	No. of cases	No. of controls	*GSTM1* null		Reason for exclusion
			OR*	95% CI*	
Kempkes et al. (110), 1996	113	170	1.81	1.1, 2.98	High prevalence of exposure to occupational bladder carcinogens; use of newborn controls
Lafuente et al. (112), 1993	75	75	2.41	1.25, 4.67	Phenotype based; restricted to smokers
Lafuente et al. (109), 1996	66	55	1.39	0.68, 2.87	Phenotype based; high prevalence of schistosomiasis
Rothman et al. (111), 1996	38	43	1.00	0.41, 2.45	Benzidine exposure
Abdel-Rahman et al. (107), 1998	37	34	2.99	1.13, 7.96	High prevalence of schistosomiasis
Anwar et al. (108), 1996	22	21	6.97	1.57, 30.87	High prevalence of schistosomiasis

* OR, odds ratio; CI, confidence interval.

subject, case-control status, *GSTM1* genotype, method used to ascertain genotype, smoking status and history, age, gender, race, alcohol consumption, and bladder tumor stage and/or grade. Second, the principal investigators of all other eligible studies were sent letters inviting them to participate in a pooled analysis by sharing the data from their relevant study (or studies). The data requested were similar to those collected by investigators of the International Collaborative Study on Genetic Susceptibility to Environmental Carcinogens. Investigators who did not respond to the letter within 8 weeks were sent a second letter further explaining the project and again inviting them to participate. Finally, in order to reduce the risk of publication bias, we contacted all participating investigators, including those who had contributed their data to the International Collaborative Study on Genetic Susceptibility to Environmental Carcinogens, and asked them to identify any analyzed but unpublished data on this topic. One study (114) was excluded because the published paper provided case data only aggregated with other smoking-related cancers and we were unable to obtain the original data. Some study data sets differed slightly from the data contained in the published studies. For both the meta- and pooled analyses, when there was more than one publication based on related data sets, we included only the most recent results.

For the meta-analysis, we included all eligible studies published in manuscript or abstract form through August 2000 that either provided a cross-tabulation of *GSTM1* genotype status by case status or for which we had the original data. We used the unpublished genotype results from one study published with only phenotype data (115) and also included results from one unpublished study (M. Romkes). When possible, all data were extracted from the published manuscript or abstract; otherwise, the necessary data were obtained from the data set. A total of 17 studies (2,149 cases and 3,646 controls) were identified and included (115–130; M. Romkes, unpublished study) (table 1). We fitted random effects models (131–133), which estimate summary measures by weighting each individual-study result by a factor of within- and between-study variance. Homogeneity of study results in different groupings was assessed via the Q statistic, with p values of <0.05 indicating lack of homogeneity. Meta-analysis can produce biased results if the included studies are a biased sample of studies in general, so we assessed publication bias via funnel plots (134), Begg's test (134), and Egger's test (135).

For the pooled analyses, we included all eligible studies for which we had the original data, including two that were unpublished (table 1). This comprised a total of 13 studies (1,935 cases and 1,444 controls, including 12 studies and 74 percent of the cases included in the meta-analysis): four in the United States (115–117; M. Romkes, unpublished study), seven in Europe (118–120, 123–125; A. K. Daly, unpublished study), and two in Asia (128, 129). Because some of the data sets we were provided contained only case data (117, 125; A. K. Daly, unpublished study), these data sets were included only in case-only analyses; the remaining 10 data sets were included in case-control analyses. We used fixed effects models with study-specific intercepts to estimate odds ratios and 95 percent confidence intervals with SAS Institute, Inc. (Cary, North Carolina) software (136). Estimates were adjusted for age (<60, 60–74, ≥75 years), gender, race (Caucasian, other), and study site. Random effects models produced results very similar to those from fixed effects models and are not presented here.

Summary odds ratios for both the meta-analysis and pooled analysis were calculated for all the studies combined as well as for subgroups of studies. Subgroups were defined by geographic region (United States, Europe, and Asia) and by explicitly defined incident versus prevalent cases: incident (115, 118, 119, 121, 123, 124) and prevalent (118, 122, 123); studies that provided data separately for both incident and prevalent cases had the data included in both of the corresponding analyses. Subgroup analyses were also performed for 1) studies whose participants were known or presumed to be Caucasian or that contained identifiable Caucasian subgroups (115–127; M. Romkes, unpublished study), 2) studies or subgroups within studies of transitional cell carcinoma (115–123; M. Romkes, unpublished study), and 3) studies published in manuscript form (i.e., excluding 119 and 128). We also attempted to examine multiplicative and additive interactions among *GSTM1* status, various smoking measures, and risk of bladder cancer. Additive interactions were assessed using a model in which the confounders were log-linear and the exposures of interest (*GSTM1*, smoking, and their interaction) were linear (137). We did not estimate absolute risks because most of the studies included in this analysis were clinic or hospital

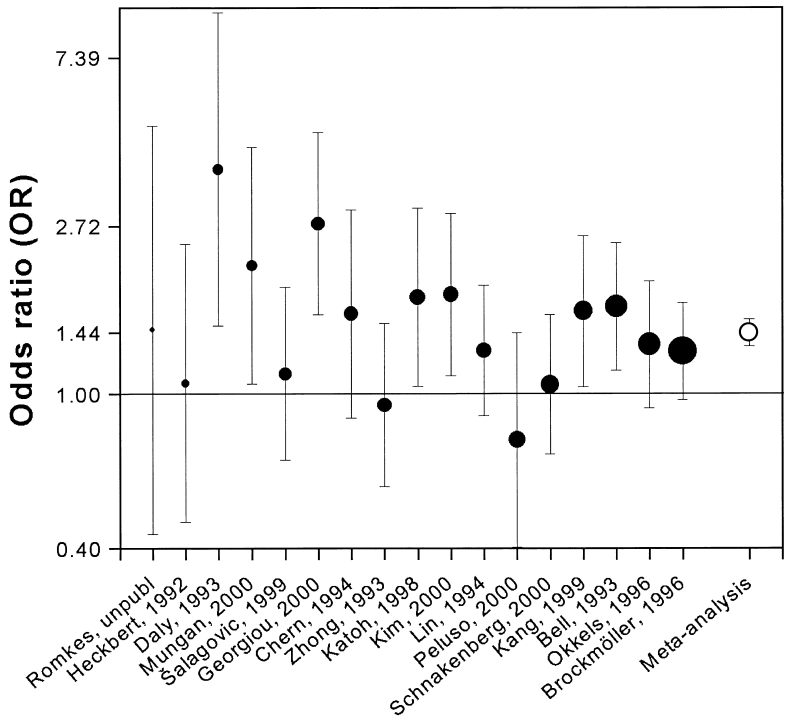

FIGURE 2. Odds ratios and 95% confidence intervals for glutathione *S*-transferase M1 (*GSTM1*) null status and bladder cancer risk. Solid circles are proportional in area to the number of cases. The vertical axis is on a log scale. Romkes, unpubl, unpublished data of M. Romkes (University of Pittsburgh, Pittsburgh, PA).

based, and it would not be appropriate to estimate baseline rates from these study controls. In addition, reliable estimates of baseline rates do not exist for some of the study populations included. Interaction analyses were performed with Epicure for Windows software (138).

Most of the studies included in our analyses used clinic- or hospital-based controls. Use of hospital-based controls can introduce bias in the assessment of gene-environment interactions when disease resulting in hospitalization among the controls is related to the exposure or gene being studied (139). Our risk estimate for the main effect of cigarette smoking on bladder cancer (odds ratio (OR) = 1.86, 95 percent CI: 1.54, 2.26) was lower than the estimates of 3.02 (95 percent CI: 1.56, 5.82) obtained for the three population-based studies in our data set and the 2.5–3.5 reported in other population-based, case-control studies of bladder cancer (23, 140–143), suggesting a biased control sample. We examined this bias by performing stratified analyses of hospital-based and population-based studies. We also assessed the magnitude of this bias using parameters estimated from external and internal information, especially the two main effects and interaction for being hospitalized. Because case-only analyses of *GSTM1* and ever smoking indicated no multiplicative interaction and there was no observed association between *GSTM1* and control disease, we examined bias only in the additive interaction parameter. Details of this method are presented in the Appendix.

Because appreciable variability in study results can arise from differences in control selection, we further examined gene-environment interactions via case-only analyses involving *GSTM1* status and smoking. In such analyses, an odds ratio is calculated from cross-classification of exposure and genetic information among cases only (144–147). A case-only odds ratio of >1 in the present study would indicate that the relation between smoking and bladder cancer is stronger among *GSTM1* null subjects than among *GSTM1* active subjects. The same study subgroups and smoking measures used for case-control analyses were used for the case-only analyses, although some case-only subgroups contained more cases than did their case-control counterparts because some of the data sets we were provided contained only case data. Because valid interpretation of the case-only odds ratio depends upon independence of the exposure and genetic factor in the underlying population, we assessed this independence via the χ^2 statistic among the associated controls.

RESULTS

Meta-analysis

Although the studies included in the meta-analysis had odds ratios for the main effect of *GSTM1* null and bladder cancer ranging from 0.76 (95 percent CI: 0.40, 1.44) to 3.81 (95 percent CI: 1.50, 9.70), all but two reported odds ratios above 1 (table 1, figure 2). Seven of the studies observed

TABLE 3. Odds ratios and 95% confidence intervals for the association of *GSTM1* null status and bladder cancer—meta-analyses

	No. of studies	No. of cases	No. of controls	*GSTM1* null cases		*GSTM1* null controls		OR*	95% CI*	*Q p* value*
				No.	%	No.	%			
All studies	17	2,149	3,646	1,264	59	1,813	50	1.44	1.23, 1.68	0.08
Asia	3	398	479	257	65	251	52	1.73	1.66, 1.81	0.95
Europe	10	1,366	1,667	781	57	822	49	1.39	1.09, 1.77	0.02
United States	4	385	1,498	226	59	740	49	1.44	1.38, 1.50	0.71
United States and Europe	14	1,751	3,165	1,007	58	1,562	49	1.38	1.15, 1.65	0.05
Incident cases only†	6	737	964	420	57	480	50	1.33	0.94, 1.88	0.03
Prevalent cases only	3	275	644	164	60	321	50	1.50	1.02, 2.20	0.21
Caucasians	14	1,707	2,514	986	58	1,250	50	1.39	1.16, 1.67	0.05
TCC* cases only	10	1,192	2,415	720	60	1,187	49	1.57	1.23, 2.01	0.05
At least 100 cases and 100 controls‡	8	1,506	2,592	899	60	1,310	51	1.42	1.26, 1.60	0.60
Manuscripts only	14	1,867	3,354	1,082	58	1,660	49	1.42	1.19, 1.70	0.03
Results based on published data from studies included in pooled case-control or case-only analyses										
All studies§,¶	11	1,620	2,691	952	59	1,345	50	1.40	1.20, 1.64	0.29
Asia	2	286	259	179	63	128	49	1.70	1.67, 1.72	0.75
Europe	6	962	1,003	555	58	513	51	1.32	1.01, 1.73	0.13
United States	3	372	1,429	218	59	704	49	1.44	1.18, 1.75	0.50
United States and Europe	9	1,334	2,432	773	58	1,217	50	1.35	1.13, 1.61	0.24
Studies included in table 4§	9	1,430	1,339	849	59	683	51	1.46	1.20, 1.76	0.21

* OR, odds ratio; CI, confidence interval; *Q p* value, *p* value of the *Q* statistic, which assesses homogeneity of study results ($p < 0.05$ suggests too much heterogeneity for pooling); TCC, transitional cell carcinoma.

† Includes only studies that explicitly indicated restriction to incident cases or identified subgroups of incident cases within studies. Studies of prevalent cases and studies that did not state whether cases were incident or prevalent are not included in these analyses.

‡ Comprised three studies from Asia, three from Europe, and two from the United States.

§ Includes the nine published studies for which the provided data sets contained both cases and controls (115, 116, 118–120, 123, 124, 128, 129).

¶ Includes the two published studies for which the provided data sets contained only cases (117, 125).

significantly elevated risk estimates. The larger studies tended to have similar risk estimates. There was appreciable overlap of confidence intervals.

Including all the study results produced a significant summary odds ratio for *GSTM1* null of 1.44 (95 percent CI: 1.23, 1.68). Statistical tests indicated no significant heterogeneity of individual study results (*Q* statistic with *p* value = 0.08) (table 3). Grouping study results by geographic region produced similar summary odds ratios for Asia, Europe, and the United States of 1.73 (95 percent CI: 1.66, 1.81), 1.39 (95 percent CI: 1.09, 1.77), and 1.44 (95 percent CI: 1.38, 1.50), respectively, although there was significant heterogeneity among European study results (*Q* statistic with *p* value = 0.02). Results from studies with at least 100 cases and 100 controls (eight studies, 1,506 cases, 2,592 controls) produced a summary odds ratio of 1.42 (95 percent CI: 1.26, 1.60) (*Q* statistic with *p* value = 0.60). Statistical tests indicated significant heterogeneity of results from the six studies that either restricted to incident cases or presented data on this subgroup (*Q* statistic with *p* value = 0.03). Grouping the published results from the 11 studies for which we had the data sets gave a summary odds ratio of 1.40 (95 percent CI: 1.20, 1.64) (*Q* statistic with *p* value = 0.29). A funnel plot (figure 3) and Begg's and Egger's statistical tests ($p = 0.43$ and $p = 0.34$, respectively) indicated no substantial publication bias.

Pooled analysis

Summary odds ratios for *GSTM1* null based on the pooled data were very similar to the summary odds ratios obtained from the meta-analysis (table 4). The adjusted summary odds ratio for all studies combined (10 studies) was 1.40 (95 percent CI: 1.20, 1.64). Examination by geographic region produced adjusted summary odds ratios of 1.70 (95 percent CI: 1.19, 2.42) for Asia, 1.29 (95 percent CI: 1.05, 1.58) for Europe, and 1.49 (95 percent CI: 1.06, 2.08) for the United States. Grouping results of the five studies having at least 100 cases and 100 controls gave an adjusted summary odds ratio (OR = 1.37, 95 percent CI: 1.16, 1.63) similar to that for

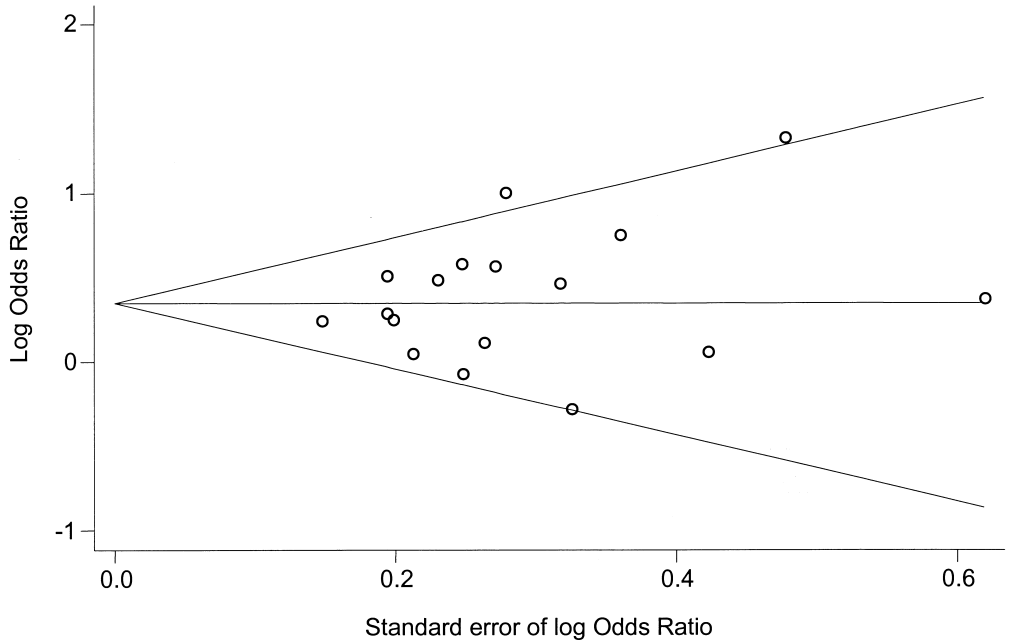

FIGURE 3. Begg's funnel plot for assessing publication bias in relation to glutathione *S*-transferase M1 (*GSTM1*) null status and bladder cancer risk.

TABLE 4. Odds ratios and 95% confidence intervals for the association of *GSTM1* null status and bladder cancer—pooled analyses

	No. of studies	No. of cases	No. of controls	*GSTM1* null cases		*GSTM1* null controls		Crude		Adjusted*	
				No.	%	No.	%	OR†	95% CI†	OR	95% CI
All studies‡	10	1,496	1,444	890	59	745	52	1.38	1.19, 1.60	1.40	1.20, 1.64
Asia	2	297	266	187	63	129	49	1.81	1.29, 2.53	1.70	1.19, 2.42
Europe	5	927	784	541	58	414	53	1.25	1.03, 1.52	1.29	1.05, 1.58
United States	3	272	396	162	60	202	51	1.41	1.04, 1.93	1.49	1.06, 2.08
United States and Europe	8	1,199	1,180	703	59	616	52	1.30	1.10, 1.53	1.33	1.12, 1.58
Incident cases only§	3	275	293	155	56	157	54	1.12	0.80, 1.56	1.21	0.85, 1.73
Caucasians	8	1,177	1,152	693	59	606	53	1.29	1.10, 1.52	1.33	1.11, 1.58
TCC† cases only	7	1,038	1,076	625	60	563	52	1.38	1.16, 1.64	1.41	1.17, 1.70
At least 100 cases and 100 controls	5	1,238	1,034	720	58	530	51	1.32	1.12, 1.56	1.37	1.16, 1.63

* Adjusted for age (<60, 60–74, ≥75 years), sex, race (Caucasian, other), and study site, except in Asia and Europe, where results are adjusted for only age, sex, and study site.

† OR, odds ratio; CI, confidence interval; TCC, transitional cell carcinoma.

‡ Includes unpublished data from nine published studies and one unpublished study (M. Romkes, University of Pittsburgh, Pittsburgh, PA).

§ Includes only studies that explicitly indicated restriction to incident cases or identified subgroups of incident cases within studies. Studies of prevalent cases and studies that did not state whether cases were incident or prevalent are not included in these analyses.

all studies combined. Most subgroup summary odds ratios ranged between approximately 1.3 and 1.5. Crude summary odds ratios were similar to adjusted odds ratios for all subgroups.

Nine studies provided sufficient information to classify subjects by smoking status (table 5). Pooling these nine studies gave a significant adjusted summary odds ratio for ever smoking of 1.91 (95 percent CI: 1.57, 2.32). The adjusted summary odds ratio for European studies was 1.73 (95 percent CI: 1.35, 2.20) and for US studies was a substantially higher 3.34 (95 percent CI: 2.18, 5.11). We did not estimate a summary odds ratio among Asian studies because only one Asian study had smoking information on both cases and controls. The pooled estimate for Caucasians (OR = 2.04, 95 percent CI: 1.65, 2.53) was similar to the all-studies' pooled estimate, as was the pooled estimate for studies with

TABLE 5. Odds ratios and 95% confidence intervals for the association of ever smoking and bladder cancer—pooled analyses

	No. of studies	No. of cases	No. of controls	Ever smokers among cases		Ever smokers among controls		OR*,†	95% CI†
				No.	%	No.	%		
All studies	9	1,485	1,360	1,204	81	938	69	1.91	1.57, 2.32
Europe	5	993	813	792	80	566	70	1.73	1.35, 2.20
United States	3	274	403	226	82	256	64	3.34	2.18, 5.11
United States and Europe	8	1,267	1,216	1,018	80	822	68	2.03	1.64, 2.51
Incident cases only‡	3	300	311	263	88	203	65	5.19	3.27, 8.26
Caucasians	8	1,245	1,187	1,001	80	805	68	2.04	1.65, 2.53
TCC† cases only	7	1,091	1,109	869	80	770	69	1.67	1.33, 2.09
At least 100 cases and 100 controls	5	1,284	1,035	1,035	81	728	70	1.83	1.49, 2.25

* Adjusted for age (<60, 60–74, ≥75 years), sex, race (Caucasian, other), and study site, except in Asia and Europe, where results are adjusted for only age, sex, and study site.

† OR, odds ratio; CI, confidence interval; TCC, transitional cell carcinoma.

‡ Includes only studies that explicitly indicated restriction to incident cases or identified subgroups of incident cases within studies. Studies of prevalent cases and studies that did not state whether cases were incident or prevalent are not included in these analyses.

at least 100 cases and 100 controls (OR = 1.83, 95 percent CI: 1.49, 2.25). Summary risk estimates for dose-response relations involving pack-years of smoking and cigarettes smoked per day are not presented because of the appreciable heterogeneity of individual study estimates.

There was no statistical evidence of multiplicative interaction, although there was a suggestion of additive interaction, between *GSTM1* and ever smoking for bladder cancer risk from the pooled case-control analyses (table 6). In stratified analyses of all studies combined, the odds ratio for *GSTM1* null status was 1.21 (95 percent CI: 0.86, 1.72) among never smokers and a significant 1.36 (95 percent CI: 1.13, 1.63) among ever smokers. The multiplicative interaction odds ratio was only slightly, and nonsignificantly, elevated (OR = 1.15, 95 percent CI: 0.79, 1.67). In contrast, the additive interaction term, which was elevated, approached statistical significance (additive interaction = 0.45, 95 percent CI: –0.03, 0.93). Estimates were similar across subgroups of studies, although for studies in the United States and Europe combined, the multiplicative interaction odds ratio increased slightly to 1.23 (95 percent CI: 0.82, 1.85), and the additive interaction term became a significantly elevated 0.53 (95 percent CI: 0.03, 1.04). When we performed analyses that also included all available data extracted from published manuscripts for which we lacked individual-level data (an additional two studies with 22 percent more subjects, for a total of 11 studies with 1,628 cases and 1,552 controls) and adjusted only for study, the multiplicative and additive interaction terms changed little, although the additive term became significant (OR = 1.14, 95 percent CI: 0.82, 1.59; additive interaction = 0.46, 95 percent CI: 0.04, 0.88, respectively). We were unable to examine pooled estimates of potential interaction by pack-years or intensity of smoking because individual study results were heterogeneous and some analyses had low statistical power.

We performed stratified analysis of hospital-based and population-based studies to assess bias in the estimate of additive interaction resulting from the use of hospital-based controls. Analysis of the six hospital-based studies in our data set (containing a total of 1,222 cases and 1,003 controls) produced an additive interaction of 0.36 (95 percent CI: –0.17, 0.89); the three population-based studies (containing a total of 135 cases and 257 controls) produced an additive interaction of 1.09 (95 percent CI: –0.57, 2.74). This difference, although modest, suggests that the observed additive interaction value of 0.45 (95 percent CI: –0.03, 0.93), based predominantly on hospital-based controls, could be an underestimate. We further examined this bias via adjustment described by Wacholder et al. (139). Based on estimates from population-based studies (23, 140–143), we considered a range of 2.5–3.5 for the risk of bladder cancer, using population-based controls, related to ever smoking among *GSTM1* active subjects (see Appendix). The adjusted additive interactions were higher than observed additive interactions across the range of odds ratios for ever smoking, varying from 0.68 (assuming a true ever-smoking OR = 2.5) to 0.86 (with a true ever-smoking OR = 3.0) and to 1.03 (with a true ever-smoking OR = 3.5).

Case-only analyses of ever smoking and bladder cancer also suggested a lack of multiplicative interaction. The case-only odds ratio for the 12 studies with *GSTM1* and smoking data (1,836 cases) was 1.08 (95 percent CI: 0.84, 1.38). The Q statistic indicated no significant heterogeneity of individual study results (p = 0.34). Subgroups of studies produced similar, nonsignificant, case-only odds ratios. Case-only analyses involving pack-years (10 studies, 1,325 cases) and cigarettes per day (eight studies, 851 cases) also provided no evidence of interaction (data not shown). The χ^2 statistics showed no association among controls between *GSTM1* and either ever smoking (p = 0.97), pack-years (p = 0.90), or cigarettes per day (p = 0.41), indicating independ-

TABLE 6. Adjusted odds ratios and 95% confidence intervals for the main and interaction effects of *GSTM1*, ever smoking, and bladder cancer—pooled analyses*,†

GSTM1 status	Ever smoker	No. of cases	No. of controls	OR‡	95% CI‡
Active	No	112	181	1	Reference
Active	Yes	454	425	1.73	1.31, 2.29
Null	No	139	195	1.20	0.86, 1.66
Null	Yes	652	459	2.37	1.80, 3.12
Multiplicative interaction				1.15	0.79, 1.67
Additive interaction				0.45	–0.03, 0.93

* Based on nine studies (1,357 cases and 1,260 controls) for which the main effect of *GSTM1* null status was an odds ratio of 1.33 (95% CI: 1.13, 1.56), and the main effect of ever smoking was an odds ratio of 1.86 (95% CI: 1.54, 2.26).

† Adjusted for age (<60, 60–74, ≥75 years), sex, race (Caucasian, other), and study site.

‡ OR, odds ratio; CI, confidence interval.

ence of the exposure and genetic factor in the underlying population, which is necessary for valid interpretation of these results.

DISCUSSION

The results of this meta-analysis suggest that persons with the *GSTM1* null genotype are at increased risk for bladder cancer, with a summary odds ratio for all studies combined of 1.44 (95 percent CI: 1.23, 1.68). The pooled analysis produced a very similar risk estimate. The similar summary risk estimates obtained using published data, pooled original data (including unpublished data), and homogeneous subgroups of published and pooled data strengthen these findings.

Results of our analyses are consistent with those observed in other related meta-analyses (100, 101). d'Errico et al. (100), in a 1996 meta-analysis of 10 studies, reported summary odds ratios of 1.54 (95 percent CI: 1.28, 1.85) for Caucasians and 2.40 (95 percent CI: 1.30, 4.45) for Asians. The higher summary odds ratio among Asians in that meta-analysis was based on only two studies. One of those studies (117) reported a highly elevated but unstable odds ratio. The other included odds ratio, obtained from an earlier report (148) of a study included in our meta-analysis (129), was unadjusted for potential confounders and was based on less well-matched cases and controls. A recent meta-analysis of 15 studies by Johns and Houlston (101), which included nine studies that were part of the current meta-analysis, reported a summary odds ratio of 1.53 (95 percent CI: 1.28, 1.84). In contrast to our analyses, which were restricted to studies of subjects whose primary risk factor for bladder cancer was likely to be tobacco smoke, the meta-analysis of Johns and Houlston included studies of subjects with known or likely occupational exposure to bladder carcinogens (110, 111), as well as subjects with a high prevalence of schistosomiasis (107–109).

We found a suggestion of additive interaction, but no statistical evidence of multiplicative interaction, between *GSTM1* null and ever smoking on bladder cancer risk. Stratified analysis and adjustment of additive interaction estimates for possible bias resulting from the use of hospital-based controls suggested that the observed additive interaction could be an underestimate of the true value. Heterogeneity of dose-response estimates across studies limited our ability to examine gene-environment interactions. Differences in the methods of obtaining detailed smoking histories may account for the variation observed when considering pack-years or cigarettes per day. The crude exposure classification represented by the ever-smoking measure may have masked an interaction between level of smoking and *GSTM1* on bladder cancer risk. Larger studies with consistent methods for ascertaining smoking history should help to clarify this issue in the future.

The pooling of original study data provided us with several unique opportunities. By combining individual efforts, we obtained a large overall sample size. We were able to adjust uniformly for confounding factors across studies. In addition, we were able to examine some gene-environment interactions that most of the original studies were too small to adequately consider.

Publication bias, if present, could bias the results of a meta-analysis or pooled analysis away from the null (149). Such bias can occur when studies with null or unexpected results are not published, leading, in most instances, to their not being included in meta-analyses. We attempted to address this issue by including in our analyses all unpublished data on this topic that we were able to identify. In addition, statistical tests indicated no substantial publication bias.

These results indicate that, among the populations studied to date, the *GSTM1* null genotype is associated with a modest increase in the risk of bladder cancer. There is a suggestion of an additive interaction between *GSTM1* and smoking on bladder cancer risk; however, neither the additive nor the multiplicative interaction parameters were statistically significant. Future studies would benefit from looking at the interaction of different levels of smoking with adequate statistical power.

LABORATORY TESTS

The methods used for determining *GSTM1* status have been described previously and will not be discussed further here. All studies included in the present analysis used genomic DNA extracted from blood. Most used polymerase chain reaction with internal control primers (116–130). Some used an enzyme-linked immunosorbent assay in addition to polymerase chain reaction (118, 119) or used Southern blot hybridization (115).

POPULATION TESTING

To date, there has been no population testing of *GSTM1* in relation to bladder cancer. GSTM1 deficiency confers only a

modestly increased relative risk and a low absolute risk. The lack of a reliable noninvasive bladder cancer screening test and the low absolute risk associated with GSTM1 deficiency limit the feasibility and usefulness of identifying GSTM1-deficient persons for bladder cancer screening purposes.

ACKNOWLEDGMENTS

The authors thank Dr. Jay Lubin, Dr. Sholom Wacholder, and Dr. Nilanjan Chatterjee for their statistical assistance.

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APPENDIX

Calculations Used to Assess Possible Bias in the Estimation of Gene-Environment Additive Interaction Resulting from the Use of Hospital-based Controls

Wacholder et al. (139) have shown how the magnitude of this bias depends on parameters that can be estimated from external and internal information, in particular the two main effects and interaction for being hospitalized. Adjusted interaction terms, presented in the text, were calculated using the following estimates:

- Odds ratio for bladder cancer, using population-based controls, from *GSTM1* null among never smokers of 1.20; obtained from our pooled analysis of all studies; based on the fact that the prevalence of *GSTM1* null among controls in this study (52 percent) was similar to the prevalence among population controls of similar ethnicity reported in other published and unpublished studies of *GSTM1* (2, 8–10);
- Odds ratio for study controls (i.e., for hospitalization for other diseases) from the *GSTM1* null among never smokers of 1.00; also based on the similar prevalence of *GSTM1* null among controls in this study and comparable population controls in other studies;

- Odds ratio ranging from 2.5 to 3.5 for bladder cancer, using population-based controls, from ever smoking among *GSTM1* active persons; obtained from other population-based studies (23, 139–142);
- Odds ratio ranging from 1.45 to 2.02 for study controls from ever smoking among *GSTM1* active persons; estimated by dividing the odds ratio for bladder cancer, using population-based controls, from ever smoking among *GSTM1* active persons (ranging from 2.5 to 3.5) by the corresponding odds ratio estimate in our pooled analysis (OR = 1.73);
- Odds ratio for the association of *GSTM1* null and ever smoking among study controls of 0.98;
- Multiplicative interaction of *GSTM1* null and ever smoking for bladder cancer, using population-based controls, of 1.13; calculated as the product of the observed multiplicative interaction, 1.15, and the association of *GSTM1* null and ever smoking among study controls, 0.98.